IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re-application of
André Claude

Group art Unit : 1651

Serial N° : 10/084,395

Examiner : Ware, D. K.

Filed : February 25, 2002

For : Process for cultivating mites, nutrient preparation for this process, and preparation of allergenic extracts from these mites.

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :

I, Thierry Batard, residing at 49, rue Joseph Chaleil / 78000 Versailles (France) ;

Declare and say :

I am citizen of France.

I am PhD, graduated from Institut National Agronomique Paris-Grignon, France

I have been working as a Research Associate in the Research & Development Department of Stallergènes (France) for 11 years.

I am an inventor of the present patent application. I am aware that the Examiner considered that, in the absence of some unexpected results, it would have been obvious to one of ordinary skill in the art, at the time the claimed invention was made, to provide a medium comprising lyophilized amino acids for mite culture.

However, it was unexpected that the provision of amino acids in lyophilized form instead of non treated amino acids, in the diet of mites, would result in a great enhancement in the yields of mite production and allergenic activity.

Actually, we found that mites cannot be cultured and grown efficiently where human scales, shrimp eggs or powdered pig's liver are merely replaced with a mixture of commercially available crystalline amino acids. As stated in the application, the yields are very low then (page 2, lines 11-15).

The experimental results shown in Figure 1 herewith further illustrate that the culturing yields are dramatically affected by the replacement of human scales by a mixture of amino acids that are not in lyophilised form.

Figure 1 displays the results of a comparative experiment of *Dermatophagoides pteronyssinus* culture in flasks, either in a medium comprising human scales, or in the same medium wherein human scales have been replaced with a mixture of crystalline amino acids ("non treated amino acids").

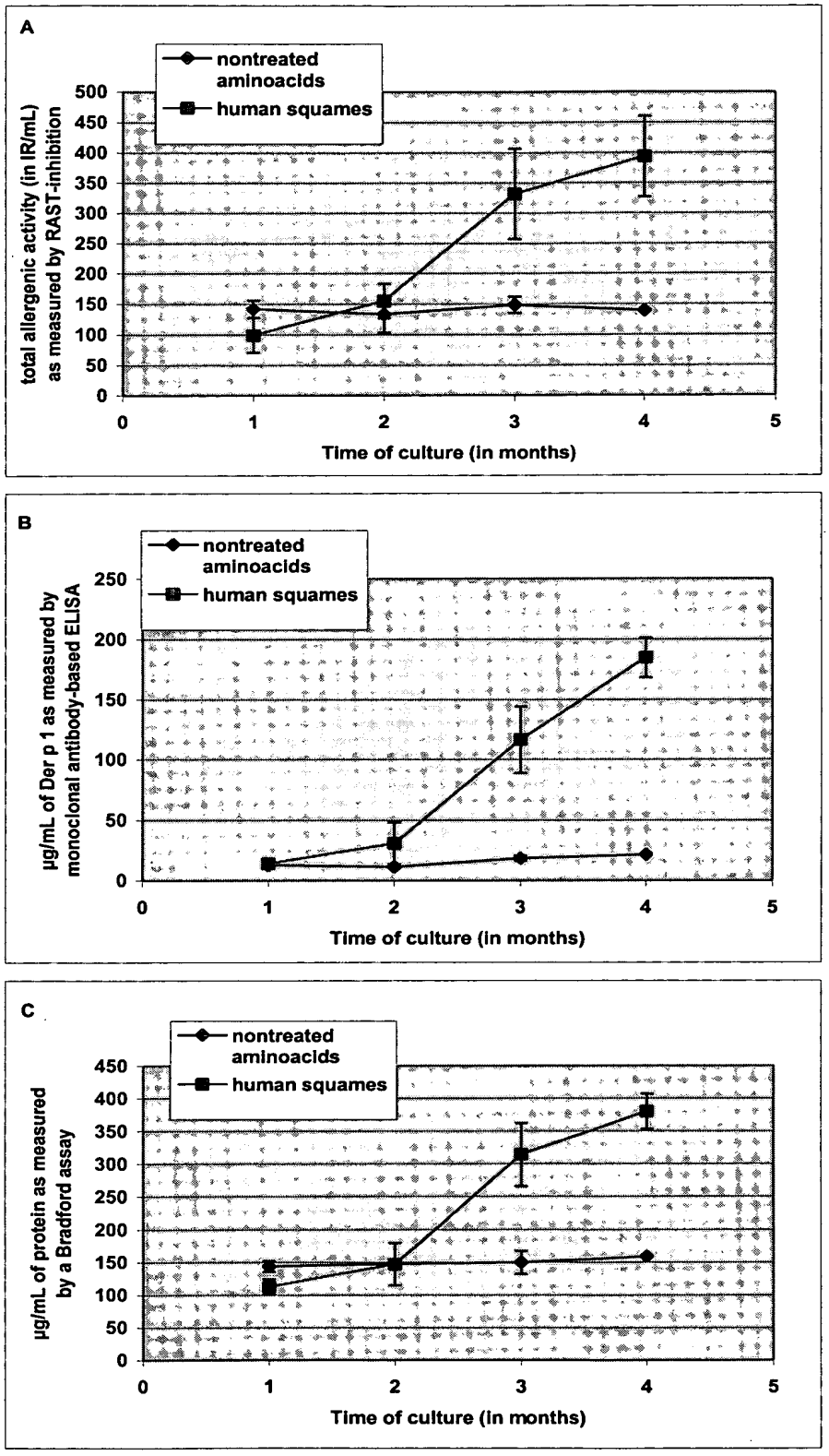


Fig. 1. Total allergenic activity (A), Der p 1 (B) and protein (C) contents in 1/20 (m/v) extracts of *D. pteronyssinus* cultured for different times on non treated crystalline aminoacids vs human scales.

Figure 1 illustrates that, in cultures carried out on a medium where human scales have been replaced with non treated amino acids, the yields either expressed in term of total allergenic activity (Fig. 1 A), of *Dermatophagoides pteronyssinus* group 1 allergen (Der p 1) concentration (Fig. 1 B), or of total protein concentration (Fig. 1 C), are dramatically reduced.

On the contrary, the results set forth in the patent application demonstrate that, where the amino acids of the mixture are in lyophilized and/or ground to a particulate size below 250 µm, no appreciable yield reduction is observed as compared with a medium containing human scales instead.

Example 2 of the patent application discloses a medium for culturing and producing mites that comprises human scales.

The patent application further describes in example 1, a medium identical to the one of example 2 except for replacement of the human scales with a mixture of lyophilized amino acids.

Comparative analysis of the media according to examples 1 and 2 have been performed during a first cycle, after 3 months of culture (Table 1), or during a second cycle of culture, after 2.5 and 3 months of culture (Tables 2 and 3, respectively).

The results shown in each of Tables 1, 2 and 3 demonstrate that a medium wherein human scales have been replaced with a mixture of amino acids that have been lyophilized and/or ground to a particulate size below 250 µm, achieves similar yields in allergenic activity, allergen concentration (Der p 1 or Der p 2) and total protein concentration.

Accordingly, it has been possible to provide a medium for cultivating and producing mites which is deprived from substances of human or animal because we worked out that these substances could be replaced by a mixture of amino acids without altering the culture yields provided the amino

acids are supplied in lyophilized form and/or particulate form of size below 250 μm.

When we started our research work, we could not anticipate that the form of the amino acids would be so determinant on the culturing and producing yields. Thus these results were unexpected to us.

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The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed April 25, 2005